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The Study of Shade Avoidance mutant *P25-52* and TAA1 Protein Dimerization

P25-52 突变体以及 TAA1 蛋白的二聚化的研究

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ABSTRACT

Light is life for plants. Presence of nearby plants results in a reduction in the red to far-red ratio (R:FR) caused by a specific enrichment in FR light reflected from the surface of neighboring leaves. In response to low R:FR ratio signals, many plants display increased elongation of stems and petioles, leaf hyponasty, reduced branching, accelerated flowering and reduced chlorophyll content. Those responses are collectively termed the "Shade Avoidance Syndrome" (SAS) provide an essential survival strategy in shade. On the other hand this phenomenon is a detrimental as it can significantly reduce yield of grain or seeds in high-density plantings typical of modern agriculture. Despite the negative effect of SAS on crops, little is known about its molecular machinery in plants. We have isolated a shade avoidance (*sav*) *P25-52* mutant, which displayed interesting characteristics such as short hypocotyl under shade, late flowering and serrated leaf. In this research, we tested the responses of *P25-52* towards different lights and hormones.

We found that the *P25-52* is shorter than wild type under simulated shade, while in dark the hypocotyl elongation is not as different as it is in shade. This indicates that *P25-52* is defective in gene required somewhat specifically for shade induced elongation growth. When we examined de-etiolation response of *P25-52* to continuous red and blue light, the results showed that the response curve of *P25-52* is similar to that of the wild type. These data indicate that defective gene in *P25-52* is not required for response to red or blue light during de-etiolation.

Results from the *P25-52* flowering time assay showed that *P25-52* flowered later with more leaves than wild-type plants; which suggest that the defective gene in *P25-52* may participate in flowering time control.

We also assessed whether *P25-52* is defective in GA, ABA and auxin hormones biosynthesis or signaling. We then found that *P25-52* appears to be hypersensitive to GA in elongation growth and to ABA in germination phenomenon, which implies that *P25-52* is not defective in GA or ABA signaling, but may be defective in their biosynthesis pathways.

Finally, we found that *P25-52* does not respond to the exogenous auxin analogue, picloram, in shade. Also the results showed that *IAA19* and *IAA29* expression level decreased significantly in *P25-52* under shade whereas the induction of *PIL1* remains the same in both mutant and wild type. It suggests that *P25-52* is not defective in auxin biosynthesis. On the other hand, when we treated *P25-52* with exogenous 1uM IAA and examined the expression of *IAA19*; *P25-52* was still auxin responsive. Thus, we suggested that *P25-52* may involve in auxin transport or shade signaling specific events.

Regarding the second part of our project, we explore the homodimerization and heterodimerization status of TAA1 protein. We found that TAA1 can directly interact with another molecule of TAA1 and form homodimer according to the results obtained by yeast two-hybrid and pull down experiments. Additionally, we have examined if TAA1 is able to form heterodimer with its homologues, and the pull down results suggested that TAA1 is able to form heterodimer with TAR2. It suggests that TAA1 protein may form and function as a dimer in *planta*.

Keywords: Shade Avoidance; Auxin; TAA1; Dimerization.

中文摘要

光是植物的生命。当植物周围有其它植物遮挡时，其它植物反射的远红光使其所感受到的红光远红光比例下降。当红光远红光比例下降时，最为显著的反应是下胚轴和叶柄快速生长，叶片偏下性，侧枝减少，开花提前，叶绿素含量降低等。这些反应统称为“避荫综合症”。避荫反应是植物的一项基本生存策略。另一方面，它也会使典型现代农业中高密度种植的作物减产, 因为体内的能量从储能器官分配到快速伸长的茎和叶柄。尽管避荫综合症对作物有不良影响，但是我们对其分子调控机制还不是很清楚。我们分离了一株避荫突变体 *P25-52*，此突变体在遮荫条件下下胚轴较短，另外开花也较晚，叶片呈锯齿状。在此项研究中，我们主要观察了在不同的光照以及激素处理条件下 *p25-52* 的反应。

我们发现，*p25-52* 在模拟遮荫条件下其下胚轴比野生型要短，而在黑暗条件下其下胚轴长度与野生型几乎没有差别。这就意味着 *P25-52* 是参与避荫反应的特异基因。我们检测了 *p25-52* 和野生型在持续红光和蓝光下的去黄化反应，结果显示当红光或蓝光光强增加时，*p25-52* 突变体以及野生型的下胚轴长度都会缩短。这个数据表明，*p25-52* 基因不参与到红光和蓝光下的去黄化反应过程中。

P25-52 开花时间的实验结果显示，*p25-52* 突变体叶片数较多，开花时间比野生型要晚，这表明 *p25-52* 可能参与到开花信号通路中。

我们也检测了 *p25-52* 是否参与到 GA, ABA,生长素的合成或信号转导途径。我们发现 *P25-52* 突变体在伸长生长方面对 GA 是超敏感的，在种子萌发方面对 ABA 也是超敏感的，这意味着 *p25-52* 并没有参与到 GA 和 ABA 的信号转导途径中，不过有可能参与到其合成过程中。

最后，我们发现 *p25-52* 突变体在遮荫条件下对外源生长素类似物毒莠定没有反应。另

外，在遮荫条件下，*p25-52* 突变体中 *IAA19* 和 *IAA29* 的表达量明显降低，而 PIL1 的表达量跟野生型相比基本不变。这说明 *P25-52* 在生长素合成过程中没有缺陷。另一方面，当我们在培养基中加入外源的 1uM 的 IAA 处理 *p25-52*，同时也检测了 IAA19 的表达量，*p25-52* 仍对生长素正常应答。因此，我们得出 *p25-52* 可能参与到生长素的运输过程或遮荫信号转导过程中。

第二部分的实验是关于 TAA1 蛋白的二聚化，我们正在探索 TAA1 的同源二聚化以及异源二聚化。通过酵母双杂交以及 Pull Down 实验我们发现，TAA1 可以直接和另外一个 TAA1 蛋白形成同源二聚体。另外，我们用同样的方法也检测了 TAA1 是否能够和其同源蛋白形成异源二聚体，结果表明 TAA1 可以和其同源蛋白 TAR2 形成二聚体。因此，在植物体当中 TAA1 蛋白可能是要先形成二聚体然后再发挥功能。

关键词：避荫反应、生长素、TAA1、二聚化

List of Abbreviations

ABA: Abscisic acid

AD: activation domain

Amp: Ampicilin

APS: Ammonium persulfate

BD: binding domain

BRs: Brassinosteroids

BSA: Albumin bovine serum

Chl: Chloramphenicol

CK: Cytokinin

DMSO: Dimethyl sulfoxide

DTT: dethilthreitol

EDTA: ethylene diaminetetra-acetic acid

GA: Gibberellins

GAF: cGMP phosphodiesterase/adenyl cyclase/Fhl1

HKRD: Histidine-kinase-related domain

IAA: Indole-3-acetic acid

IAM: Indole-3-acetamide

IAOx: Indole-3-acetaldoxime